Overview of the Hemostasis Research Program: Advances and Future Directions[1]

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ABSTRACT

The mission of the Combat Casualty Care Research Program of the US Army Medical Research and Material Command is to reduce the morbidity and mortality resulting from injuries on the battlefield through the development of new life-saving strategies, surgical techniques, biological and mechanical products, and the timely use of telemedicine technologies. One of the major areas of focus of the Combat Casualty Care Program is hemorrhage control. This article provides an overview of the Hemostasis Research Program and its accomplishments to date, and makes suggestions for areas of basic research in the future. The Hemostasis Research Program is based at the US Army Institute of Surgical Research (ISR) and includes collaborations with extramural research laboratories.

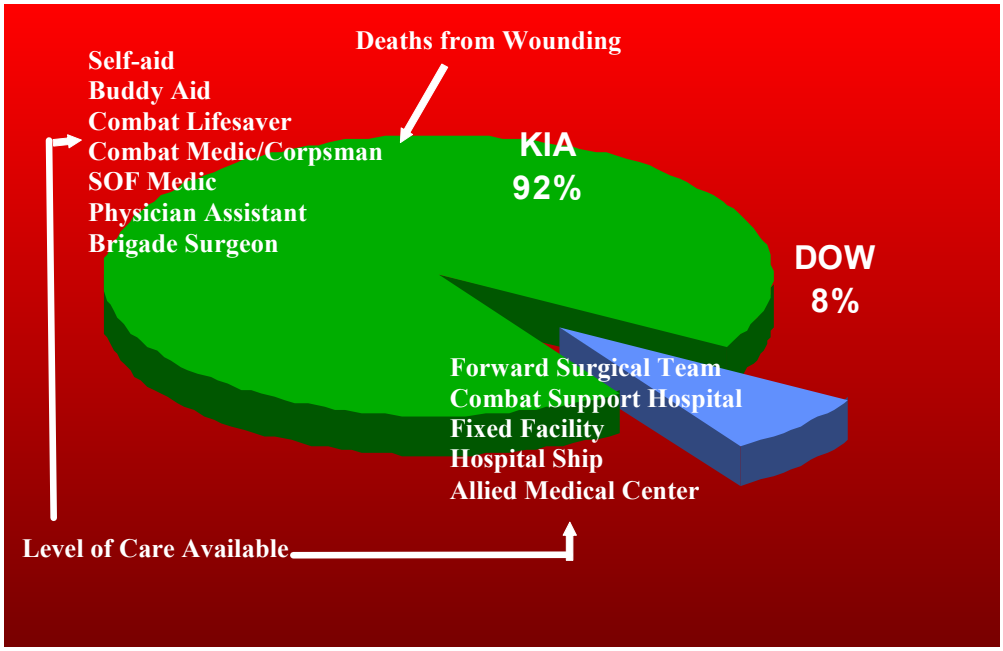

Figure 1: Level of care available pre-and post-evacuation. Adapted from [1].

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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1.0 THE NEED: DECREASED COMBAT MORTALITY

Due to improvements in survival after evacuation from the battlefield, overall mortality from combat wounds has decreased during the past hundred years. Although mortality has decreased for those dying of wounds after evacuation (DOW), there has been little improvement in the number of those killed in action (KIA) (Figure 1). Furthermore, the percentage of the wounded that die on the battlefield increases with prolonged evacuation [1], an increasingly likely scenario under the Objective Force as troops are more dispersed throughout the combat arena.

Hemorrhage is the leading cause of death from wounds on the battlefield (accounting for over 50% of deaths) [1] and the second leading cause of death in civilian trauma [2]. Although some soldiers killed on the battlefield are clearly unsalvageable and become KIA within minutes of impact, it appears that approximately one-third of KIA would be salvageable (Figure 2) with the development and fielding of new methods for early intervention [3]. Data supporting the position that a salvageable population of KIA exists were obtained in Oman in 1973 [4] and Panama in 1989 [5]. In both instances, the stationing of emergency medicine physicians at casualty collection points provided advanced medical care closer to the point of wounding and resulted in lower KIA rates than in previous conflicts. Given the possibility of a significant population of salvageable KIA, the hemorrhage control program has focused primarily on providing new methods, drugs, or devices to those present or near the point of wounding, such as the wounded soldier himself, his buddy, the combat lifesaver, or the combat medic.

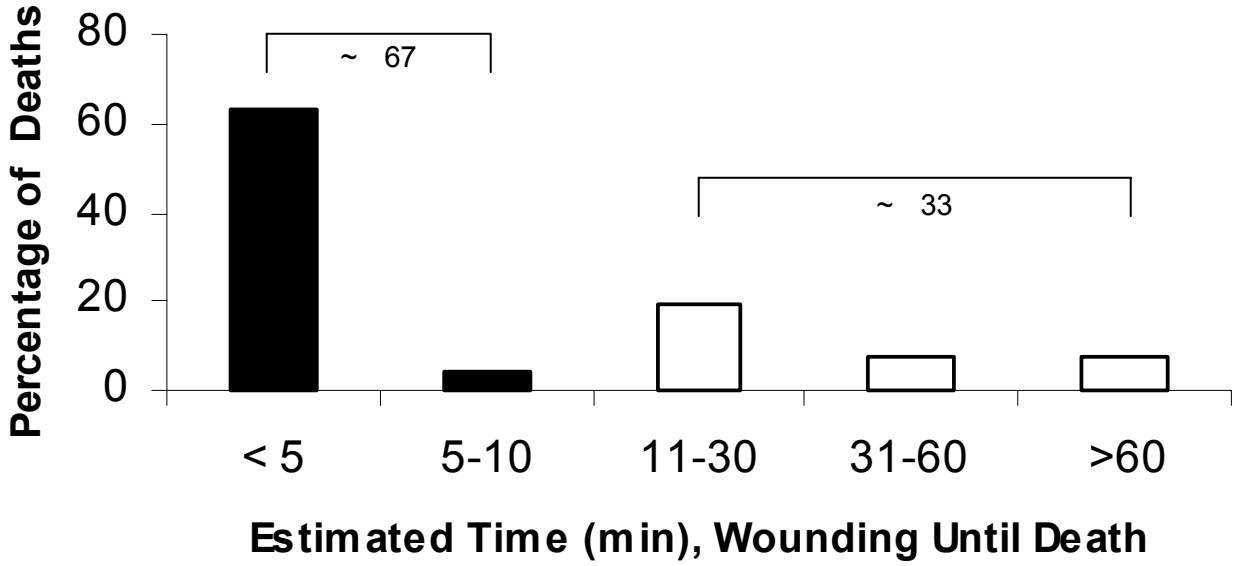

Figure 2. Percentage of deaths on the battlefield occurring at different times from wounding in Viet Nam. Making the assumption that deaths occurring within the first 15 minutes are not salvageable, approximately 33% of KIA deaths are potentially salvageable. Adapted from [3].

2.0 HEMOSTASIS RESEARCH PROGRAM OBJECTIVES

Figure 3 depicts the current state of field hemorrhage control and the objectives we strive to meet by 2010. Approximately 20% of hemorrhagic deaths are due to compressible wounds (i.e., those that are accessible to direct pressure), treatable with pressure dressings, tourniquets, and mechanical surgical methods. However, the vast majority (approximately 80%) of hemorrhagic deaths on the battlefield are due to intracavitary hemorrhage, which is not accessible for direct compression (e.g., within the pelvic, abdominal or thoracic cavities). Currently, no method other than surgical intervention can treat intracavitary hemorrhage. The mission of the Hemostasis Research Program is to develop procedures, devices or agents that may be used by the soldier himself, a buddy, a combat medic or higher echelon medical personnel to control compressible and non-compressible hemorrhage under far-forward situations. We will therefore discuss in subsequent sections the devices, drugs, and methods that are currently being developed or evaluated to accomplish this objective.

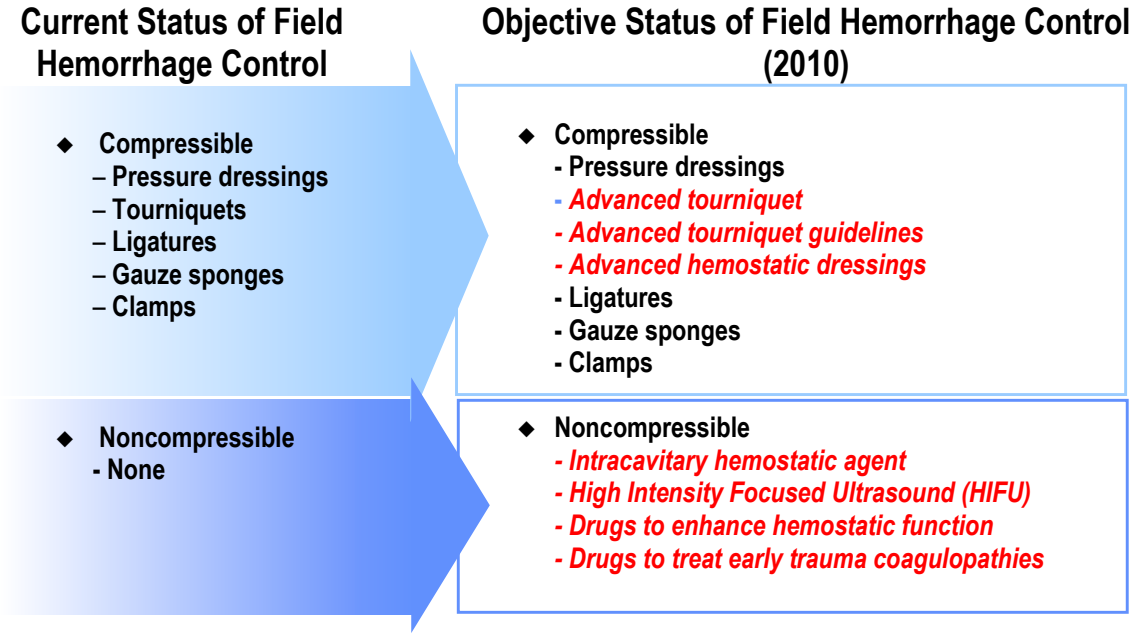

Figure 3. Current (2004) and future (2010) field hemorrhage control.

2.1 One-Handed Tourniquet

One need identified by the soldier in the field has been a tourniquet that can be self-applied by a wounded soldier with one hand. Project development was originally funded by the US Special Operations Command (USSOCOM), but was subsequently "handed off" to the Hemostasis Research Program for further development. To this end, Calkins *et al.* [6] developed a needs survey that asked Special Operations corpsmen to rank desired characteristics in a tourniquet. A comprehensive search of commercially available tourniquets or patented tourniquet designs was subsequently performed and several novel tourniquet designs were also developed. These tourniquet designs were then evaluated as to how well they met the desires of the user community as expressed in the survey, as well as how well they performed under austere far-forward conditions. Special Operations corpsmen then tested seven tourniquet designs for successful tourniquet placement and time required for placement. Preliminary field-testing results suggested a ratchet design as the best tourniquet available for field use. It was later recognized that this was not the optimal design [6].

Subsequently, two new prototype designs (cinch and wrap) were developed and modifications were made to the ratchet design by investigators at the Walter Reed Army Institute of Research (WRAIR). These three designs were then evaluated based on their effectiveness, application time, size and weight. Furthermore, laboratory testing on artificial limbs assessed the pressure distribution resulting from the tourniquet application. An *ad hoc* panel containing both scientists and combat medics then convened at the ISR, and this panel concluded that the cinch design best met the requirements of the user community. With slight modifications, this tourniquet was produced and sent to field users (USSOCOM, AMEDD Center and School, US Navy and Marine Corps) for ongoing evaluation. Subsequently, a manufacturer was found and large-scale manufacturing was begun.

Special Forces medics in Afghanistan and Iraq have used the one-handed tourniquet. Additionally, an expert panel was convened to promulgate guidelines for field use of tourniquets as well as to address the potential for field removal of the tourniquet if used in combination with advanced hemostatic dressings. Researchers within the Soft Tissue Research Area at ISR are currently investigating the ability to enhance tissue salvage utilizing tourniquets with further improvements.

2.2 Advanced Topical Hemostatic Dressings

Under the auspices of the Hemostasis Research Program, a variety of topical hemostatic dressings have been tested for their potential applications to trauma using both severe liver injury and arterial injury models in swine [7-9]. As a result of these evaluations, two dressings were approved for external use in the field, the American Red Cross Dry Fibrin Sealant Hemostatic Dressing (under an IND protocol) and the FDA-approved HemCon Chitosan bandage. The ability of these bandages to act as the primary hemostatic mechanism for internal use (in lieu of surgical intervention) over prolonged periods of time (e.g., in the event of extended evacuation) is currently being tested at the ISR. For a detailed description of the development and evaluation of these dressings, please see the paper by Kheirabadi *et al.*

2.3 Intracavitary Hemostatic Agent

A major issue to be addressed by the Hemostasis Research Program is a method to control bleeding from non-compressible truncal wounds, for which surgical intervention is currently the only effective treatment. The concept of an intracavitary hemostatic agent was advanced to treat such an injury in the field. The hypothesis was that a hemostatic material could be infused into a closed body cavity by a trocar, spread throughout the closed cavity, and interact with the bleeding sites to stop hemorrhage. In the gunshot wound, for example, foam could be delivered through the hole left by the penetrating object and administered close to the injury site to reduce internal bleeding. In order for this to be a viable option, however, a putative intracavitary hemostatic agent must: 1) produce hemostasis without applied pressure when administered directly to an actively bleeding site; 2) distribute uniformly throughout the body cavity when it is introduced into a closed cavity and stop hemorrhage upon contact with actively bleeding tissues. In addition to providing hemostatic efficacy, it has been proposed that an intracavitary hemostatic agent might also provide enough intracavitary pressure to provide some level of tamponade, thereby aiding in hemorrhage control. It is important to emphasize, however, that intracavitary pressure cannot be increased to the point at which function of vital organs is compromised.

Initially, several liquid hemostatic agents were tested in internal hemorrhage models in small animals to evaluate their ability to provide hemostasis without applied pressure. Surgical liquid fibrin sealant (FS), for example, was transformed into a foam and sprayed directly onto the bleeding surface of a lacerated liver in rodents. Although an encouraging decrease in blood loss was observed, application of the FS foam did not

increase survival rate 1 hour post-application [10]. Furthermore, experimentation with the same formulation of FS foam in a rabbit model of partial liver resection could not replicate the reduction in blood loss seen in the rat liver injury model (Kheirabadi, unpublished data).

In subsequent work, the formulation of the active components of FS was substantially modified to provide a highly adhesive fibrin foam that would attach firmly to rabbit liver slices even if the tissues were covered with fresh blood. In rabbits, direct application of this modified FS foam on the bleeding surface of the lacerated liver was partially effective in reducing the hemorrhage and improving survival. Infusion of the FS foam into a rabbit model of closed-abdomen bleeding, however, had only a marginal effect in reducing hemorrhage or improving survival rate. In other studies using a variety of means of making the foam (e.g., chemical, propellant gas), FS foam did not prove to be effective in reducing bleeding under practical application circumstances (i.e., without producing large increases in intracavitary pressure that disrupted vital organ function).

Currently, we are in the process of testing other potential hemostatic agents that might be used as intracavitary agents. Although not a foam, FloSeal (Baxter Biomedical) consists primarily of collagen and thrombin. When applied directly to the bleeding liver injury in the open abdomen rat model, FloSeal was able to reduce blood loss by almost 25%, but did not significantly increase survival time. In a closed-abdomen model of internal hemorrhage, however, FloSeal neither reduced blood loss nor improved survival time in rats.

There are two major obstacles to the effectiveness of any such agent when infused into a closed body cavity. The first is the inability of these agents to distribute thoroughly in the cavity and reach the entire injured surfaces before they become activated. The second major obstacle is the inability of these agents to penetrate through the pooled blood around the organs and move against free-flowing blood to interact with the injured tissues themselves. Hence, although intracavitary foams/liquid agents offer an alluring solution to hemorrhage from non-compressible wounds, there are as yet many obstacles to overcome and it is unclear at this point whether this potential solution to non-compressible hemorrhage is possible using current technology.

2.4 Hemostatic Drugs

As indicated above, there is currently no treatment available to the field medic for severe non-compressible hemorrhage. One approach to this problem is to identify a drug(s) that could be administered intravenously that might act systemically to decrease bleeding. The concept of using intravenous drugs to enhance or augment the body's innate clotting mechanisms during situations in which blood loss is expected is not new. Indeed, drugs have been used in the treatment of bleeding complications for over 30 years. For example, the FDA-approved drugs epsilon-amino caproic acid, tranexamic acid, aprotinin, and desmopressin (DDAVP) have been used to reduce bleeding complications and blood loss in a variety of clinical situations including cardiac surgery, hepatic surgery, oral surgery, knee and hip arthroplasty, and in patients with bleeding disorders [11]. More recently, recombinant factor VIIa (rFVIIa) has been used very effectively in hemophiliac patients for controlling acute bleeding episodes and during surgical procedures [12].

Another objective of the Hemostasis Research Program is to screen these FDA-approved drugs for their potential use in non-compressible traumatic hemorrhage. Preliminary results suggest that epsilon-amino caproic acid, tranexamic acid, aprotinin, or desmopressin (DDAVP), when used alone or in combination, neither decreases bleeding time nor increases survival time following severe liver injury in rodents (Ryan et al., in preparation).

A more promising candidate to provide hemostasis following trauma is rFVIIa. A growing number of case reports document the successful use of rFVIIa to decrease blood loss in trauma patients [13]. In laboratory models using pigs with normal coagulation function, however, rFVIIa does not appear to decrease blood loss following severe liver injury, aortic injury, or liver avulsion [14-17]. Administration of rFVIIa in these pigs does, however, activate clotting mechanisms and increases the pressure at which rebleeding occurs during resuscitation, suggesting that rFVIIa may strengthen the nascent clot [15, 16]. In pigs with abnormal coagulation function induced by reduction of body temperature (hypothermia) and dilution of blood (hemodilution), rFVIIa decreased the severe blood loss following severe liver injury in two studies [18, 19] but not in another [20]. It therefore appears that rFVIIa use may be beneficial in the coagulopathic soldier in whom bleeding cannot be stopped by other means. Ongoing laboratory investigations will further delineate the conditions under which this drug might be useful.

In addition to rFVIIa, we are studying the ability of a new drug, Factor Xa-PCPS (phosphatidyl choline-phosphatidyl serine vesicles), to reduce bleeding in swine models of trauma. We have established collaborative agreements with investigators at Haematologic Technologies, Inc. and the University of Vermont, who developed the drug . In preliminary laboratory tests, this drug stopped cuticular bleeding in both normal and hemophiliac dogs [21]. As other new hemostatic agents are developed, they will likewise be tested in trauma-relevant animal models for their potential to decrease bleeding and to save the lives of wounded soldiers on the battlefield.

2.5 Mechanisms of Early Trauma-related Coagulopathy

As alluded to above, early responses (within 24 hours) to trauma and subsequent resuscitation, especially under battlefield conditions, may include hypothermia, hemodilution and acidosis. Such conditions induce coagulopathies in which normal coagulation function is altered and disrupted. As part of the Hemostasis Research Program, we are investigating trauma-related coagulopathy in a complex setting designed to more closely model combat injuries sustained on battlefields. Our goal is to identify the changes of coagulation activity, platelet function, and fibrinolysis using our existing animal model and techniques, as well as to develop new techniques and understandings of the basic physiological mechanisms underlying the development of coagulopathy.

We are currently studying physiological mechanisms underlying the development of such coagulopathies. In our initial project, we have developed an *in vivo* swine model to study coagulopathy with hypothermia and acidosis, as well as other *in vitro* methods using physiologically relevant agonists. One of these *in vitro* methods is a measurement in minimally altered pig blood that illustrates the clot process over time. We have found that acidosis and hypothermia (alone and in combination) cause significant increases in bleeding time and decreases in fibrinogen concentration and platelet level. These were associated with significant increases in prothrombin time (PT) and activated partial thromboplastin time (aPPT), which are measures of clotting via the extrinsic and intrinsic coagulation pathways, respectively. However, platelet function was unchanged when temperature was decreased from 39 to 32°C. Development of this *in vivo* animal model and new *in vitro* methods has expanded our ability to characterize the coagulopathic state in greater detail than that provided by previous investigations. These initial results have provided valuable information about the mechanisms underlying coagulopathy induced by hypothermia and acidosis, and will be expanded to include investigations into coagulopathies associated with hemodilution or combinations of this trauma-induced triad (hypothermia, acidosis and hemodilution). Our comprehensive approach is aimed towards gaining insightful information and directions for pharmaceutical intervention to correct trauma-related coagulopathies and thereby save lives of wounded soldiers.

2.6 High Intensity Focused Ultrasound (HIFU) Device

In addition to screening potential hemostatic agents, the ISR is currently evaluating other means to reduce non-compressible hemorrhage. Ideally, a combat medic or other care provider would have a device to non-invasively visualize a source of internal bleeding and to safely cauterize it. Although this sounds like science fiction, investigators at the National Center for Physical Acoustics at the University of Mississippi and the Applied Physics Laboratory at the University of Washington are working toward such a device. These investigators are developing a hand-held device that will 1) incorporate a computerized Doppler system to locate bleeding structures and 2) focus sound waves to cauterize the bleeders without damaging the overlying tissues [22]. So far, acoustic hemostasis has been shown to be effective in sustaining hemostasis (up to 60 days) in exposed splenic lacerations [23]. Currently, these investigators have developed animal models in which blood vessel and liver injuries are made non-invasively. These injuries are subsequently visualized and cauterized using non-invasive methods. The Hemostasis Research Program has a collaborative agreement with the Applied Physics Laboratory, thereby leveraging industry and academic investments to meet Army needs. Investigators from the two programs interact on a regular basis and HIFU will eventually be tested in animal models of trauma at the ISR.

3.0 INTEGRATED APPROACHES

It should be emphasized that the hemostatic tools discussed above are not conceived as disparate solutions to the problem of bleeding; rather, the concept is that these tools can be used to complement each other in the field. Figure 4 depicts two scenarios in which the use of hemostatic tools could be integrated. In the first, a soldier with a bullet wound to the thigh that includes femoral artery injury could be treated at the level of self/buddy aid by applying a tourniquet, an advanced hemostatic dressing or both. If the tourniquet accomplishes vascular control, an advanced hemostatic dressing might then be applied and the tourniquet released, thereby improving the possibility of limb salvage. The battalion surgeon might then use HIFU to cauterize the arterial injury, if necessary. In the second scenario, a soldier with a shrapnel wound that produces severe liver laceration could be treated by a combat medic using an intracavitary hemostatic agent and/or an intravenously administered hemostatic drug. The battalion surgeon might then opt to use HIFU to cauterize the wound and, if the soldier had developed coagulopathy, additional intravenous agents might be administered to reverse the coagulopathy. The goal of the Hemostasis Research Program is to provide an array of advanced hemostatic tools to care providers, thereby allowing greater flexibility in patient treatment based on immediate clinical/combat demands.

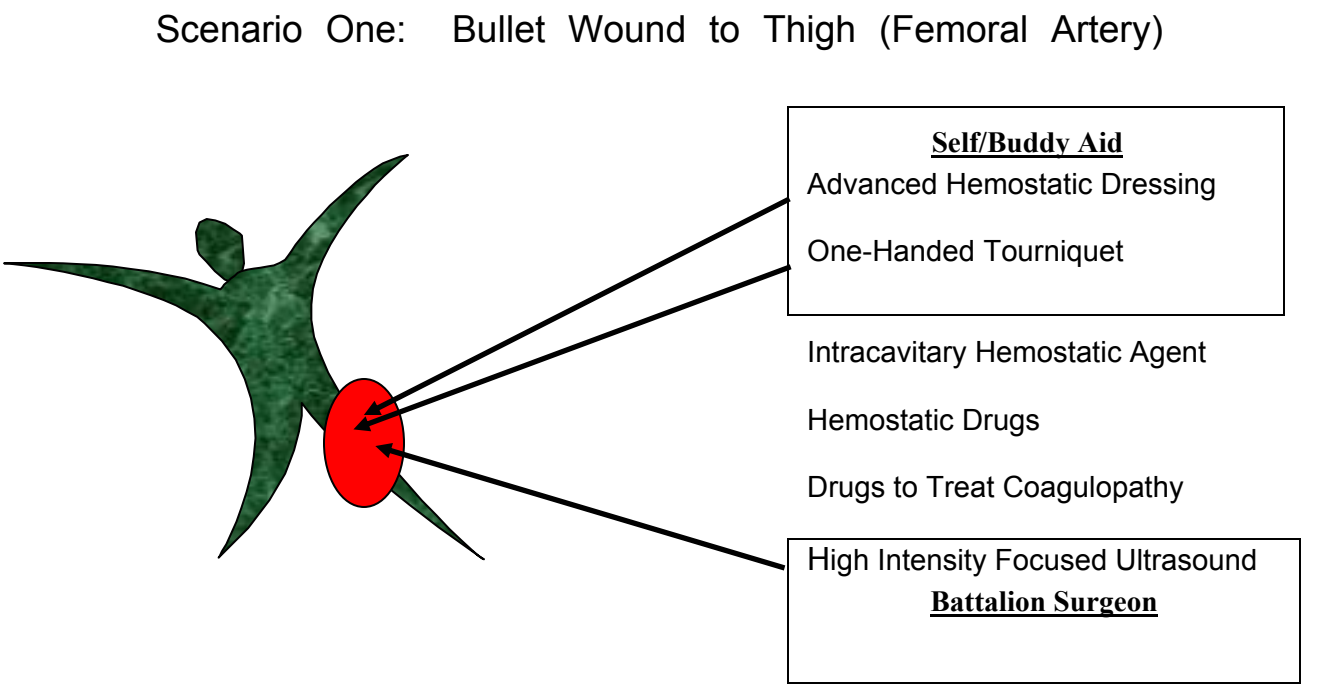

Figure 4. Possible scenarios for integrated use of hemostatic tools. Current pre-evacuation treatment for scenario one is gauze dressing and use of field expedient tourniquet or belt. For scenario two, there is currently no efficacious pre-evacuation treatment available.

4.0 FUTURE DIRECTIONS

As the Army moves toward the Objective Force and the troops are more dispersed on the battlefield, units must be more self-sustaining in terms of all support requirements, including medical needs. Evacuation of casualties may be prolonged, requiring the need for medical care through a progression of post-traumatic states over a period as long as 96 hours. A scenario can be envisioned, for example, in which the casualty first needs enhanced hemostasis and then needs treatment for hypothermic and acidotic coagulopathy. Next, the patient may progress to a hyperfibrinolytic state, a state of inappropriate intravascular activation of the coagulation system, or a septic state. Superimposed upon these conditions may be ischemia-reperfusion injury due to resuscitation or a massive inflammatory response. Other confounding factors on the future battlefield that may impact coagulation function include the use of artificial oxygen carriers (blood substitutes), immune modulators, performance enhancers or other such drugs. The ability to manipulate the hemostatic mechanisms and the way they interact with other systems will therefore be critical to optimize the potential for recovery and survival of the casualty.

Before we can manipulate the hemostatic mechanism, however, we must first understand how the coagulation system responds to trauma and how it impinges upon other systems such as the immune system. In essence, we must better understand the integrated physiological responses to traumatic injury to guide us in the subsequent development of life-saving strategies. For example, it is currently known that thrombin generation (which is necessary for coagulation) activates the inflammatory system, which can lead to detrimental tissue effects subsequent to hyperinflammation. Modulation of this activation at the level of the endothelial cell may thus allow us to increase coagulation function while decreasing inflammation. As another example, there is very little information currently available about how physiological states that soldiers routinely experience (e.g., dehydration, sleep deprivation, cold stress, heat stress, acute exercise or combinations of these) affect the coagulative response to subsequent trauma. Although no data currently exist that specifically address the effects of these physiological factors on trauma responses, related studies of elite athletes suggest that such stressors may have a profound effect on trauma-related hemorrhage and we see an urgent need to expand on this knowledge base. Finally, when tissue responses to the ischemia produced by blood loss are more fully understood, it may also be possible in the future to supplement hemostatic mechanisms by simultaneously manipulating such responses either through conventional pharmacological means or by new techniques such as gene therapy. Thus, the need for more basic research to understand the physiological mechanisms underlying the response to trauma exists and will continue to grow. The Hemorrhage Research Program will move to fill this need by performing both clinical and laboratory research to understand the interactions between physiological systems and their response to trauma. Further understanding of the basic mechanisms underlying coagulation and subsequent physiological responses will provide future solutions that not only reduce bleeding in the short term but will also decrease long-term detrimental effects. Our vision is to continuously improve upon hemostatic tools so that the KIA rate in future battles will progressively decrease.

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